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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re the Application of: McKENZIE et al.

Serial No.: 09/163,089

Filed: September 29, 1998

Atty. File No.: 4102-1

For: "COMPOSITIONS FOR IMMUNOTHERAPY AND USES THEREOF"

Group Art Unit: 1645

Examiner: Zeman, Robert A.

DECLARATION OF DR. GEOFFREY A PIETERSZ (Under 37 CFR 1.132)

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I, Geoffrey A Pietersz, declare as follows:

1. I am a co-inventor of the above-referenced patent application and am familiar with the application. I am a skilled artisan in the field of immunology/chemistry.

2. This Declaration is being submitted in conjunction with an Amendment and Response to the Office Action having a mailing date of October 23, 2001.

3. The following discussion is provided in traverse of the Examiner's rejection of Claims 1, 3-17, 19-21, 26, 38 and 70 under 35 U.S.C. § 112, first paragraph.

4. With regard to the Examiner's position that the specification is not enabling for conjugates in which the antigen is other than MUC1, I submit that a skilled artisan, using the guidance in the instant specification, would be able to produce a conjugate containing other antigens, and which would be immunogenic.

More specifically, the skilled artisan would be able to produce a carbohydrate polymer containing an antigen other than MUC1 by first synthesizing a polymeric chain wherein the carbohydrate monomers may include mannose or any one or a mixture of carbohydrate monomers such as those disclosed on page 28, lines 1-10 of the present specification. This carbohydrate polymer could be activated and conjugated to the antigen as disclosed at pages 28, line 16 to page 32, line 9.

Annexure 1 to this Declaration contains experiments which I have conducted using various antigens that are n t MUC1, *viz* the E7 antigen from the *Papilloma* virus, hen egg Ovalbumin (OVA), and a model protein antigen containing three CTL epitope peptides corresponding to three antigens – ovalbumin, papilloma virus, E7 and Mucin 1. As can be seen, the data show that conjugation of these antigens in accordance with the present specification are immunogenic in that cytotoxic T lymphocyte immune responses are elicited upon exposure to the conjugates.

In summary, the specification provides sufficient evidence that antigens other than MUC1 may be conjugated in the manner described, and that such conjugates can be used to increase cytotoxic T cell responses.

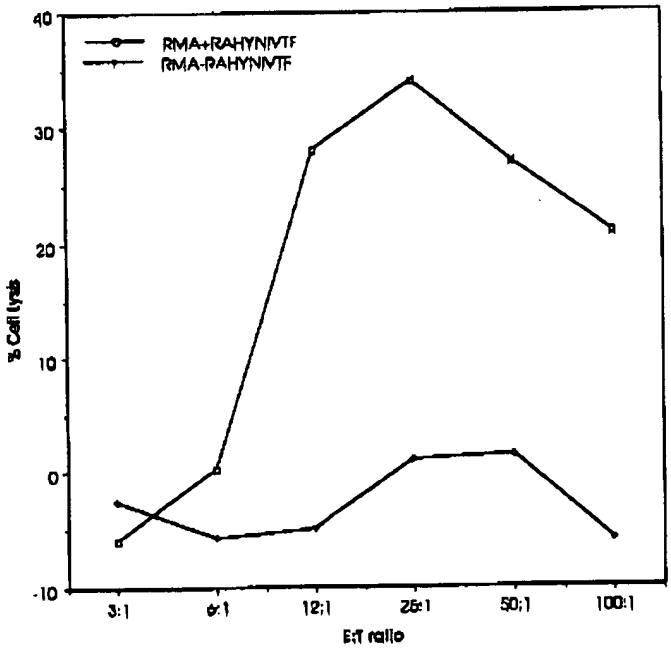
5. I hereby declare that all statements made herein of my own are true and that all statements made on information and belief are believed to be true; and further that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the subject application or any patent issuing therefrom.

Date: 25/3/02 By: G.A Pietersz
Geoffrey A Pietersz

ANNEXURE 1

1. Immune responses to Mannan-GST- E7

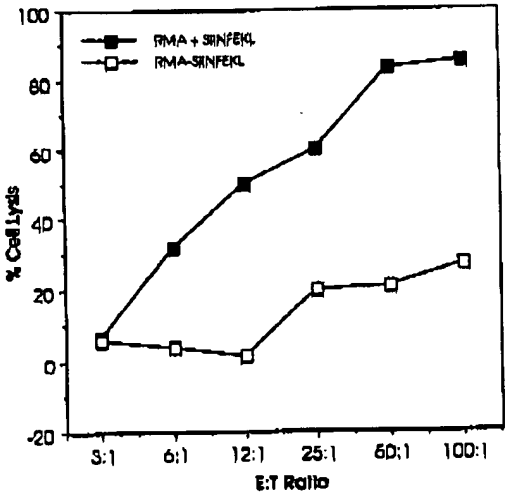
E7 is a human *Papilloma* virus antigen has the CTL epitope sequence: RAHYNIVTF. A fusion protein, GST-E7 was conjugated to mannan as described in the present application, and used to immunise C57BL/6 mice on days 0, 10, 17. Ten days after the last immunisation spleen cells were collected and used for CTL assay as described, using RMA cells pulsed with the E7 CTL peptide (RAHYNIVTF), or unpulsed cells, as targets.



As shown in the figure above, CTLs generated in mice immunised with mannan-E7 specifically and significantly killed or lysed RMA cells pulsed with the E7 epitope (RMA+RAHYNIVTF) compared to unpulsed cells (RMA-RAHYNIVTF), which were largely unaffected. The data indicate that immunisation with mannan-E7 stimulates an immune response (CTL) to the viral antigen.

2. Immune responses to Mannan-Ova

Mannan was conjugated to Ovalbumin (Ova) as described in the present application, and used to immunise C57BL/6 mice and tested as described above.



As shown in the figure above, CTLs from mice immunised with Mannan-Ova (RMA + SIINFEKL) selectively killed RMA cells pulsed with the Ova CTL peptide. By comparison, unpulsed target cells (RMA-SIINFEKL) were not affected or lysed.

3. IMMUNE RESPONSES TO A MODEL PROTEIN(GST-Ova/E7/MUC1)

To ascetain if cellular responses are generated *in vivo* to any tumour associated antigen when conjugated to mannan a model protein antigen was genetically engineered. The DNA encoding for the model protein containing several CTL peptide epitopes (Ova K^b, E7 D^b and MUC1 K^b) was prepared using splice overlap extension PCR and the oligonucleotide duplex was introduced into the BamH1/EcoR1 site of pGEX-2T. The nucleotide and amino acid sequence is shown below:

```
1/1                                31/11
GGA TCC GGA AAG TCG ATA ATC AAC TTT GAG AAG TTA GGT AAA CGT GCT CAT TAT AAT ATT
G  S  G  K  S  I  I  N  F  E  K  L  G  K  R  A  H  Y  N  I
61/21                              91/31
GTT ACA TTT GGT AAG ACC TCG GCC CCG GAC ACC AGG CCG GCC CCG GGC TCC GGC AAA GAA
V  T  F  G  K  T  S  A  P  D  T  R  P  A  P  G  S  G  K  E
121/41
TTC ATC GTG ACT GAC TGA CG
F  I  V  T  D  *
```

The protein was expressed and purified by GSH affinity chromatography as described in the present application. It was then conjugated to mannan and used to immunise C57BL/6 mice as described in the present application.

C57BL/6 mice were immunised with 5µg of the fusion model protein on days 0, 10, 17 and cellular responses were measured by CTLp as described. Groups of mice were also immunised with Mannan-Muc1 and Mannan-E7.

The target cells were pulsed with the stimulators shown below. Cp13-32 is the VNTR epitope of Muc1. T4N1 was used as a control. The results are given in the right hand column.

Immunogen	Stimulator	CTLp Frequency (Target RMA + Peptide)
Mannan-GST-Ova/E7/MUC1	Cp13-32	1/12,800
	E7 9 mer	1/73,200
	Ova 9mer	1/15,000
	T4N1	Not detected
Mannan-GST-MUC1	Cp13-32	1/8,000
Mannan-GST-E7	E7 9 mer	1/48,000

The above data indicate that immune responses can be generated to each of the epitopes within the model protein. The responses to E7 and Muc1 following immunisation of the model protein were similar to that obtained by immunisation of GST-E7, or GST- MUC1, conjugated to mannan. Thus, immunisation of animals with a multiple-antigen construct conjugated to mannan can stimulate the immune response to each of the composite antigen.

* * *